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CENTRAL FAX CENTER****OCT 19 2007****Amendments to the Specification:**

Please replace the paragraph beginning at line 4 of page 15 with the following:

Figure 6: Visualization of hPygo2 and β -catenin knockdown in EOC cell lines by confocal microscopy. In the colour version of this Figure, red fluorescence indicates expression of β -catenin and green fluorescence indicates hPygo2 expression.

Please replace the paragraph beginning at line 9 of page 17 with the following:

(d) (e) Human Pygopus protein is expressed in malignant cancer cell lines independently of Wnt-signalling factors. Total protein was extracted from eight different cell lines representing four different tumor types and processed for Western analysis using antibodies against Wnt target proteins and transducers, as well as anti-hPygo2 antibodies.

Please replace the paragraph beginning at line 24 of page 17 with the following:

Figure 11: Knockdown of β -Catenin in Mcf-7 cells by RNAi. Reagent control (the transfection agent Oligofectamine™ Oligofectamine) and non-specific siRNA controls are indicated.

Please replace the paragraph beginning at line 7 of page 18 with the following:

Figure 12: Knockdown of endogenous hPygo2 mRNA and protein using antisense ON performed in HeLa cells. Reagent control (Oligofectamine Oligofectamine™), antisense Xenopus Pygopus2 (non-specific), and four base mismatch (mismatch) controls are indicated. Levels of cDNA and protein were standardized with GAPDH and β -Actin. Experiments were performed in triplicate.

Please replace the paragraph beginning at line 19 of page 18 with the following:

Figure 13: Knockdown of hPygo2 in Mcf-7 cells using antisense ONs. Reagent control (Oligofectamine Oligofectamine™), antisense Xenopus Pygopus2 (non-specific), and four base mismatch (mismatch) controls are indicated.

Please replace the paragraph beginning at line 1 of page 19 with the following:

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Figure 14 (A and B): Knockdown of hPygo2 in Mcf-7 cells with siRNA. Reagent control (Oligofectamine Oligofectamine™), Non-specific control siRNA (NS), β-Catenin and hPygo2A and hPygo2D siRNAs are indicated. Cell growth and knockdown of protein was assayed at 72 hrs after transfection. Results indicated are based on three experiments performed in triplicate.

Please replace the paragraph beginning at line 16 of page 19 with the following:

Figure 16: Knockdown of endogenous hPygo2 using antisense ON in HeLa cervical cancer cells. Reagent control (Oligofectamine Oligofectamine™), antisense Xenopus Pygopus2 (non-specific), and four base mismatch (mismatch) controls are indicated.

Please replace the paragraph beginning at line 4 of page 75 with the following:

(b) RNA extraction and Northern Blotting: Total cellular RNA was extracted using RNeasy the RNA purification kit RNeasy™ Mini Kit (Qiagen, QP, CANADA). Northern blot analysis was performed using a 32p-dCTP-labelled cDNA probes in Rapid-Hyb buffer at 65° C for one hour and washed to high stringency in 0.1 X at 65° C for 15 minutes as described (Lake, B.B. & Kao, K.R. Pygopus is required for embryonic brain patterning in Xenopus. Dev. Biol. 261, 132-148 (2003)).

Please replace the paragraph beginning at line 6 of page 77 with the following:

(e) Immunofluorescence: Cells were plated onto glass chamber slides and fixed in 4% paraformaldehyde for 30 minutes, rinsed in Phosphate buffered saline (PBS) twice, followed by 0.2% triton-X100 in PBS (tPBS) for 10 minutes. Cells were blocked in 10% normal donkey or goat serum and incubated overnight at 4°C with primary antibodies in 1.5% normal serum in PBS. The next day the slides were washed for 30 to 40 minutes in 0.2% tPBS and incubated for 30 minutes at room temperature with biotinylated donkey anti-rabbit (Amersham) or Cy3 donkey anti-mouse (Jackson ImmunoResearch Laboratories, Inc.) secondary antibodies in 1.5% normal sera. After a 30 to 40 minute wash in 0.1 – 0.2% tPBS, cells were incubated in streptavidin fluorescein (Amersham) in 1.5% normal sera/PBS for 30 minutes, washed in 0.1-0.2% tPBS 30 to 40 minutes mounted in 10% glycerol/PBS or

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~~Vectashield~~ the mounting medium Vectashield™ (Vector Laboratories, Inc.) and viewed under confocal microscopy using filters optimized for fluorescein and Cy3.

Please replace the paragraph beginning at line 5 of page 78 with the following:

5 X 10⁴ of SK-OV-3 cells and 6 X 10⁴ of NIH-OVCAR-3 cells were seeded into 12-well plates 24 hrs before transfection. 100-200nM of 19 mer oligonucleotides containing 3 phosphorothioated bonds(*) at each terminus (5'-G*G*C*TGAGCAAATCGTT*G*G*G-3'; Hpy5) from the coding region (nt no. 807-825) of hPygo2 (SEQ ID NO:1) and its mismatched sequence (5'-G*C*C*TGAGCTAATCATT*G*G*T-3'; SEQ ID NO:20) or anti-*Xenopus* pygo2 oligonucleotides (5'-T*T*T*GCGCCGTTCTT*C*T*C-3'; SEQ ID NO:21) was transfected into NIH-OVCAR-3 using the Oligofectamine Oligofectamine™ Transfection Kit (Invitrogen, CA, USA) and SK-OV-3 using Effectene Effectene™ Transfection Kit (Qiagen, QP, CANADA). The culture medium was changed after 24 and 48 hours transfection.

Please replace the paragraph beginning at line 13 of page 79 with the following:

Transfection of siRNA was performed as for the antisense ON, using either Oligofectamine Oligofectamine™ or RNAiEasy (Qiagen). Cells were washed in PBS 6 hours after transfection of 100nM of siRNA and replaced with fresh medium, followed by an additional transfection of 100nM of siRNA 24 hours later. The cells were then fixed and stained with propidium iodide and counted for DNA content using a Fluorescence Activated Cell Sorter.

Please replace the paragraph beginning at line 28 of page 86 with the following:

Based on our immunoblot data (Fig. 4a), β-catenin is expressed in both SKOV-3 cells and OVCAR-3 cells. Demonstration of nuclear co-localization of hPygo2 with β-catenin, therefore, would provide evidence that hPygo2 is involved in canonical Wnt signaling in EOC cell lines. Studies using conventional fluorescence microscopy may be confounded by high autofluorescence levels and low resolution associated with conventional fluorescence microscopy. So we used confocal microscopy to unambiguously visualize the normal expression and knockdown by ONs and siRNA of hPygo2 and β-catenin in SK-OV-3 and OVCAR-3 EOC cell lines *in situ* (Fig. 6. In the colour version of this Figure, not shown, red

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fluorescence indicates expression of β -catenin and green fluorescence indicates hPygo2 expression). In the untreated (Fig. 6a, e, i) and control transfected (Fig. 6 c, g) cells, β -catenin was localized to the plasma membrane, whereas hPygo2 was always concentrated in nuclei. In cells transfected with both antisense ONs and siRNA specific for hPygo2, however, the concentration of hPygo2 in nuclei was noticeably reduced (Fig. 6d, h, l). In these cases β -catenin was still often expressed and continued to be localized at points of cell-to-cell contact (Fig. 6d, h). Interestingly, knockdown of β -catenin in the SK-OV-3 cells resulted in a slight dispersion of hPygo2 protein from nuclei, but the cells clearly continued to overexpress hPygo2 (Fig. 6k). Thus, the lack of co-localization of hPygo2 and β -catenin suggests that the activities and functions of these proteins are not coupled in EOC cell lines.

Please replace the paragraph beginning at line 24 of page 91 with the following:

(d) Immunocytochemistry and Immunohistochemistry: For immunofluorescence analysis, Hs-574-mg, Bt-474 and Mcf-7 cells were fixed in 4% paraformaldehyde (30 minutes) rinsed in PBS twice and 0.2% triton-X 100/PBS (tPBS) for 10 minutes. Cells were blocked in 10% normal donkey/goat serum prior to an overnight incubation with primary antibodies in 1.5% normal serum/ PBS. After a 30 to 40 minute wash in 0.2% tPBS, cells were incubated 30 minutes with secondary antibodies in 1.5% normal sera. For hPygo-2, biotinylated donkey anti-rabbit (Amersham) and for β -Catenin, Cy3 donkey anti-mouse (Jackson ImmunoResearch Laboratories, Inc.) was used. After a 30 to 40 minute wash in 0.1 – 0.2% tPBS, cells were incubated in streptavidin fluorescein (Amersham) in 1.5% normal sera/PBS for 30 minutes. Cells were washed in 0.1-0.2% tPBS 30 to 40 minutes before mounting in 10% glycerol/PBS or Vectashield VectashieldTM (Vector Laboratories, Inc.). Images were collected using confocal microscopy (Olympus).

Please replace the paragraph beginning at line 14 of page 92 with the following:

(e) Antisense oligos and siRNA: Antisense oligonucleotides (Invitrogen) against hPygo2 were designed to contain three phosphorothioate bonds at each terminus as indicated by asterisks to enhance nuclease resistance. The sequences used were as follows: hPygo2 antisense oligo; 5'-G*G*C*TGAGCAAATCGTT*G*G*G (1Ipy5; SEQ ID NO:9), Xenopus Pygo2-specific control oligo; 5'-T*T*T*GCGCCGTTCTT*C*T*C SEQ ID NO:21, 4 base

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mismatch oligo; 5'-G*U*C*TGAGCUAATCATT*G*G*T (mismatches underlined; SEQ ID NO:28). All oligonucleotides were designed avoiding G quartets and repeated CG sequences which may result in non-specific antisense effects. β -Catenin siRNA and non-specific control siRNA were purchased as a β -Catenin siRNA/siABTM (siRNA knockdown) Assay Kit (Upstate). hPygo2 siRNA was synthesized using the (Xeragon-Qiagen) sense sequences: Hpy2A; 5'-r(CGAUGACCAGGAUGCCAUU)dTT-3' (SEQ ID NO:15) Hpy2D; 5'-r(CCAGCCUCUGGGUCAAAAC)dTT-3' (SEQ ID NO:18).

Please replace the paragraph beginning at line 11 of page 93 with the following:

All transfections utilized Oligofectamine OligofectamineTM (Invitrogen) as per the manufacturer's instructions, replacing the growth media every 24 hours. hPygo2 antisense/control oligonucleotides were transfected to a final concentration of 250 nM and all siRNAs were transfected at a final concentration of 100nM. siRNA was transfected again 24 hours after the first transfection. For RT-PCR analysis, cells were seeded at a density of 1.5 x 105 cells/well in six-well plates and were harvested 24 hours after transfection for RNA extraction. For western analysis cells were seeded at a density of 105 cells/well in twelve-well plates and were harvested 48 hours after transfection. For cell growth analysis cells were seeded in triplicate at a density of 7.5 x 104 cells/well in twelve-well plates, and were counted 48 and 72 hours after transfection using trypan blue exclusion (Sigma) with a hemacytometer.

Please replace the paragraph beginning at line 28 of page 97 with the following:

As in the HeLa cells, transfection of the (hpy5) ON into Mcf-7 cells resulted in a significant knockdown in hPygo2 protein levels as compared to the non-specific and mismatch control ONs while β -Catenin levels remained unaltered (Figure 13a). Most significantly, there was a considerable reduction of Mcf-7 cell growth (Figure 13b) after transfection with the hPygo-specific ON, as compared to the cells transfected with the non-specific and mismatch control ONs. Cell numbers were reduced to 52%, as compared to 93% with the non-specific control and 83% for the mismatch control as compared to the reagent (oligofectamine OligofectamineTM) control. These results demonstrate that the ability of the hpy5 oligonucleotide to knockdown hPygo2 protein is specific and results in a reduction in cell

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proliferation. This reduction in cell number was accompanied by a decrease in the cell cycle regulatory protein Cyclin D1 (Figure 13a), implying that the reduction of cell growth is likely due to cell cycle arrest. These results demonstrate that hPygo2 is required independently of β -Catenin in the growth of Mcf-7 cells.

Please replace the paragraph beginning at line 3 of page 102 with the following:
Figure 16 shows knockdown of endogenous hPygo2 using antisense ON in HeLa cervical cancer cells. Reagent control (Oligofectamine Oligofectamine TM), antisense Xenopus Pygopus2 (non-specific), and four base mismatch (mismatch) controls are indicated. Knockdown of hPygo2 by antisense ON results in a decrease of HeLa cell numbers 48 and 72 hours after transfection. Cell number was assayed for by direct counting with a hemacytometer using trypan blue exclusion. RT-PCR analysis shows specific knockdown of hPygo2 mRNA without effecting expression of the related Pygo family member, hPygo1. RT- is the negative control, without reverse transcriptase. Western blot analysis shows knockdown of endogenous hPygo2 protein. Levels of cDNA and protein were standardized using GAPDH and beta-Actin. Experiments were performed in triplicate.